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# Comparing the impact of chemically produced and biosynthesized zinc oxide nanoparticles on Klebsiella pneumoniae isolated from the inflammatory middle ear

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***ABSTRACT***

***Introduction:*** *Chronic suppurative otitis media (CSOM) is a sustained infection of the middle ear marked by ongoing otorrhea and inflammation, frequently linked to microbiological agents such as Klebsiella pneumoniae. The increasing prevalence of antibiotic resistance requires alternate antimicrobial approaches, including nanoparticle-based therapies. Zinc oxide nanoparticles (ZnO-NPs) have emerged as attractive options due to their extensive antibacterial characteristics. This study sought to evaluate the antibacterial effectiveness of chemically produced and biosynthesized ZnO nanoparticles (utilizing Aloe vera extract) against K. pneumoniae isolated from individuals with chronic suppurative otitis media.*

***Material and methods:*** *An in vitro experimental research was performed on isolates of K. pneumoniae derived from middle ear discharge samples of individuals with chronic suppurative otitis media (CSOM). ZnO nanoparticles were produced with both chemical techniques and green biosynthesis utilizing Aloe vera extract. Nanoparticles were analyzed utilizing UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), and energy-dispersive X-ray spectroscopy (EDX). The antimicrobial effectiveness was evaluated by the agar well diffusion method at different doses (25–200 µg/mL). Data analysis was conducted utilizing SPSS version 25.0.*

***Results:*** *K. pneumoniae was isolated in 18% of 50 clinical samples. Both varieties of ZnO nanoparticles exhibited typical peaks about 370 nm and exhibited nanometric crystallite sizes of 28 nm for chemically manufactured and 35 nm for biosynthesized variants. SEM and TEM demonstrated a spherical shape for chemically produced ZnO nanoparticles and a heterogeneous composition (spherical and rod-like) for biosynthesized nanoparticles. FTIR and EDX verified the existence of functional groups and organic residues in biosynthesized particles. Antimicrobial tests demonstrated that chemically produced ZnO-NPs displayed markedly larger zones of inhibition at all doses in comparison to biosynthesized variants ($p < 0.05$).*

***Conclusion:*** *Both chemically manufactured and biosynthesized ZnO nanoparticles have antibacterial properties against K. pneumoniae, with the chemically synthesized variants showing superior inhibitory effects. Biosynthesized ZnO nanoparticles offer advantages in terms of environmental sustainability and potential biocompatibility. These findings support the ongoing exploration of nanoparticle-based therapies as adjunctive or alternative solutions to conventional antibiotics in the management of CSOM.*

***Keywords:*** *Aloevera, CSOM, Effect zinc, Nanoparticles Biosynthesized*

## INTRODUCTION

A persistent, long-term infection of the middle ear without an intact tympanic membrane is referred to as chronic suppurative otitis media, or chronic otitis media. This condition is characterized by persistent inflammation of the mastoid cavity and middle ear. Chronic or persistent otorrhea through a ruptured tympanic membrane that lasts for two to six weeks is the hallmark appearance. [1] The characteristic symptoms of chronic suppurative otitis media are caused by disruptions in middle ear aeration and pressure equilibration, which are caused by dysfunction of the Eustachian tube. [2]

Individual with chronic suppurative otitis media are frequently afflicted by bacteria, despite the fact that viruses are the most frequent cause of otitis media. Usually, the cause is polymicrobial. Staphylococcus aureus is the most prevalent bacterium in this disorder (MRSA). Additional pathogens that can cause the illness include Pseudomonas aeruginosa, Proteus species, Klebsiella species, Bacteroides species, and Fusobacterium species. Aspergillus and Candida species, which are more commonly seen in immunocompromised people, are less common. [3] The effectiveness of traditional antibiotics against common infectious diseases has been diminished by overuse and frequent usage, necessitating a number of medical procedures to prevent this condition.[4] Significant clinical issues have arisen as a result of bacteria and fungi becoming more resistant to traditional antibiotics, particularly in cancer and AIDS patients. Furthermore, it is challenging and expensive to identify and treat microorganisms that are resistant to antibiotics.[5] By enhancing the therapeutic effect of currently available nanotechnology-based antimicrobial vector drugs, biocompatible nanomaterials offer potential strategies to prevent drug resistance. Known as nanoparticles, these materials have unique chemical and physical properties, including their incredibly small size (between 1 and 100 nm), which can be controlled, and their large surface area in relation to size, high interactivity, and functional structure.[6] Nanoparticles' antibacterial qualities allow them to evade conventional resistance mechanisms like enzyme disruption, decreased membrane permeability, target site modification, increased flow due to over-expression of Efflux pumps, and resistance to effective antimicrobials .[7] Hence the present study was done with an aim to compare the antimicrobial effect of biosynthetic by aloe Vera and chemical (commercial) zinc nanoparticles on K. pneumoniae isolated from otitis media patients.

## MATERIAL AND METHODS

The present comparative in vitro experimental study was conducted for a period of one year at department of otorhinolaryngology & head and neck surgery of Dr DY Patil medical college, hospital and research centre to assess the antimicrobial effect of zinc oxide nanoparticles (ZnO-NPs) produced chemically and biosynthesized against Klebsiella pneumoniae that was isolated from individuals suffering from chronic suppurative otitis media (CSOM). Prior to sample collection, all subjects provided written informed consent, and the institutional ethics committee granted ethical clearance. Aseptic samples of middle ear discharge were obtained using sterile cotton swabs from 50 patients diagnosed with chronic suppurative otitis media in the outpatient department. The materials were cultivated and examined microscopically for diagnosis. Screening and cultural attributes, include colony growth on various medium (nutrient agar, blood agar, MacConkey agar, eosin methylene blue (EMB) agar, and mannitol salt agar). The proliferating bacteria were characterized by their morphology, dimensions, pigmentation, margin, hemolytic behavior, and lactose fermentation; biochemical assays were additionally conducted to confirm the attributes of the isolated bacteria. The tests encompassed an indole test for indole production, a methyl red test to assess sugar fermentation and acid generation, a Voges-Proskauer test for acetone complex identification, a citrate utilization test to evaluate citrate as a sole carbon source and sodium carbonate composition, a urease test indicating urea hydrolysis and ammonium composition, an oxidase test for cytochrome production, catalase and fermentation tests for sugars, a motility screening test, and a coagulation test utilizing two methods based on previously established protocols.

### Preparation of Aloe Vera suspension

10 g of the plant's dry powder was added, along with 100 ml of non-ionic water in place of hot distilled water, and the mixture was refrigerated for 24 hours before being shaken for an additional 24 hours. The extract was removed and added to the zinc oxide solution at a concentration of 1 mM after being filtered through many layers of gauze to remove the plant's larger components and then through Whatman No. 1 filter paper.[8]

### Biological formation of zinc oxide nanoparticles

After combining 10 milliliters of aloe vera extract with 90 milliliters of 1 mM zinc oxide and bringing the mixture's pH down to 7, two laboratory vials were added—one with the extract only and no zinc

oxide, and the other with zinc oxide only and no plant extract as a control—and the flask was placed on a magnetic stirrer for 35 minutes. The white precipitate that formed at the flask's bottom was collected, cleaned three times with non-ionic water, centrifuged at 10,000 revolutions per minute for 10 minutes, and then dried in a convection oven set at 105° C for five to six hours until the precipitate dried to obtain secondary zinc oxide minutes. [9]

The electro-kinetic properties of the zinc oxide nanoparticles were evaluated using Scanning Electron Microscopy (SEM) and Transmission electron microscopy (TEM), while the zinc oxide nanoparticles were thoroughly examined using UV-Vi spectroscopy, Atomic Force Microscopy (AFM), Fourier transform infrared spectroscopy (FT-IR), Zeta Potential, and Energy-dispersive X-rays of zinc nanoparticles (EDX).

SPSS version 25.0 was used to examine the data. According to the data distribution, the Student's t-test or Mann-Whitney U test were used to compare the antibacterial activity of chemically and biosynthesized ZnO-NPs. P-values less than 0.05 were regarded as statistically significant.

## RESULTS

### Isolation and diagnosis

The results indicated that among the 50 samples with chronic suppurative otitis media, 88% exhibited bacterial growth, and Klebsiella pneumoniae was successfully identified and confirmed in 18% samples. Identification relied on colony morphology, Gram staining, biochemical assays, and was then validated by the VITEK® 2 system.


**Figure 1 Biosynthesis of Nano Zinc Oxide by Aloe Vera Extract. CA- Zinc oxide solution with aloe Vera plant, B- Zinc oxide solution, - Aloe Vera plant extract zinc oxide solution B- Zinc oxide solution**

### Biosynthesis of ZnO Nanoparticles

The extract of the aloe vera plant served as a reducing agent and stabilizer in the production of zinc oxide nanoparticles. The study's results indicated the formation of a precipitate at the base of the reaction vessel, serving as proof for the synthesis of zinc oxide nanoparticles.

### Characterization of ZnO Nanoparticles (figure 1)

- UV-Vis Spectroscopy: Both nanoparticle varieties demonstrated pronounced absorption peaks near 370 nm, so validating the production of ZnO.
- XRD analysis indicated that the peaks belonged to the hexagonal wurtzite structure of ZnO. The crystallite size was approximately 28 nm for chemically produced ZnO nanoparticles and approximately 35 nm for biosynthesized ZnO nanoparticles.
- SEM Imaging: Chemically produced ZnO nanoparticles displayed homogeneous spherical morphologies, while biosynthesized nanoparticles demonstrated a more heterogeneous morphology characterized by irregular spherical and rod-like features.
- FTIR Spectroscopy: Functional groups including –OH, C=O, and Zn–O linkages were detected, particularly signifying the presence of phytochemicals in biosynthesized ZnO nanoparticles.

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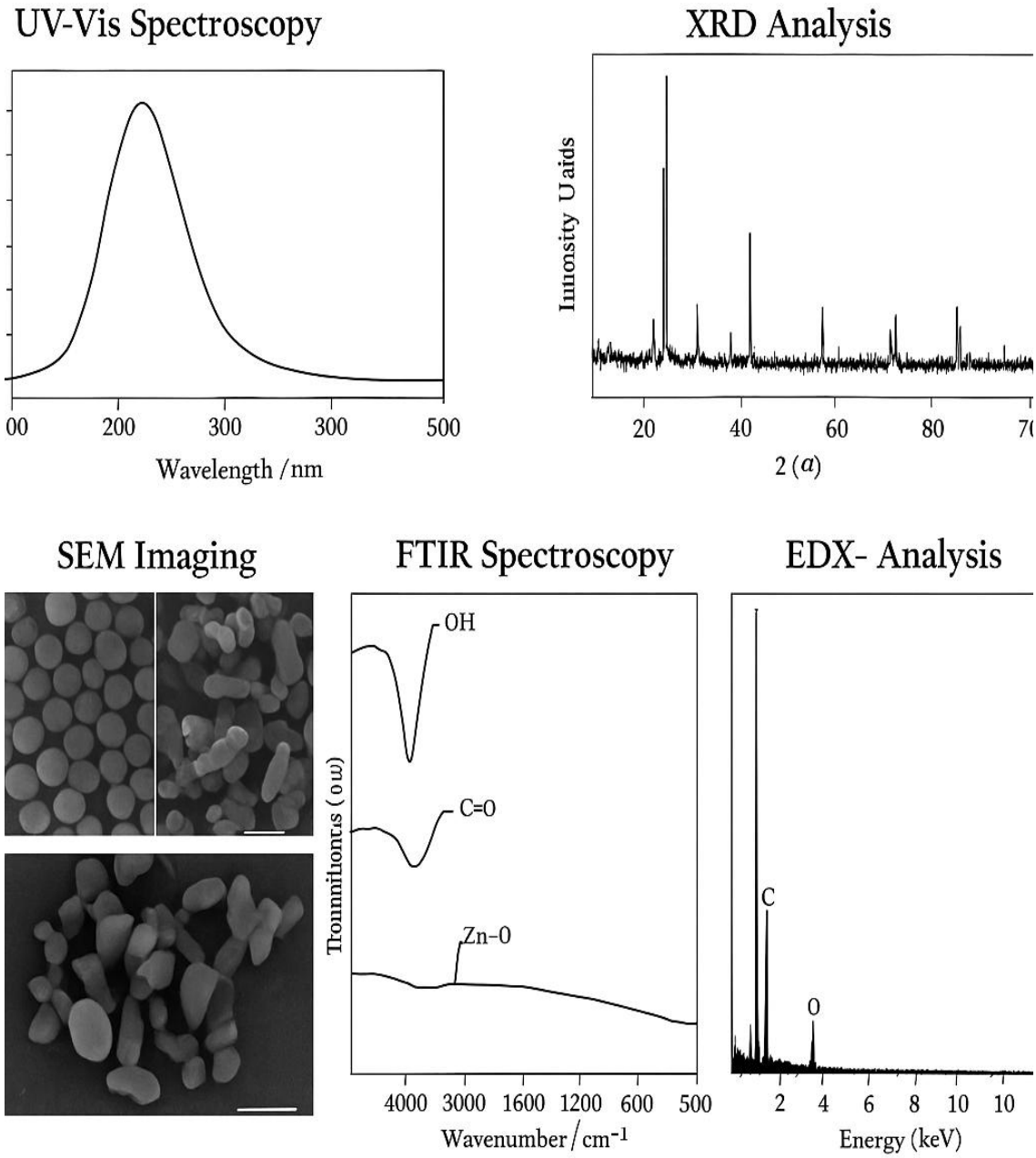
- EDX Analysis: Verified the existence of zinc and oxygen, with supplementary elements (C, N) identified in biosynthesized ZnO-NPs, presumably originating from plant metabolites.
- TEM- The prepared zinc nanoparticles are within the Nano scale and have a spherical shape.




**Inhibitory activity of commercial and biosynthetic zinc nanoparticles on K. pneumonia**

Chemically synthesized ZnO-NPs showed significantly larger zones of inhibition compared to biosynthesized ZnO-NPs at all tested concentrations ($p < 0.05$). (Table 1)

**Table 1 -Inhibitory activity**

| Concentration (µg/mL) | Mean Zone of Inhibition (mm) ± SD | Commercial Synthesized ZnO-NPs | Biosynthesized ZnO-NPs |
|---|---|---|---|
| 25 µg/mL | 7.2 ± 1.1 | 8.1 ± 0.9 | 7.2 ± 1.0 |
| 50 µg/mL | 10.5 ± 1.3 | 11.4 ± 1.0 | 10.2 ± 1.1 |
| 100 µg/mL | 13.8 ± 1.4 | 14.9 ± 1.3 | 13.3 ± 1.2 |

| | | | |
|---|---|---|---|
| 200 µg/mL | 17.4 ± 1.6 | 18.8 ± 1.5 | 16.7 ± 1.3 |

## DISCUSSION

Chronic suppurative otitis media (CSOM) is a persistent infection of the middle ear cleft characterized by ear discharge and varying levels of hearing impairment.[10] Numerous investigations on chronic suppurative otitis media (CSOM) have reported a predominance of Gram-negative bacteria in ear discharge, with Pseudomonas and Proteus species being the most frequently detected aerobic organisms, whilst Bacteroides & Peptococcus/Peptostreptococcus spp. are the most regularly isolated anaerobes. Gram-positive bacteria, such as Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA), are present but are very uncommon.[11] Reports have indicated the presence of atypical organisms, including Mycobacterium TB, M. chelonae, M. avium complex, Actinomyces species, and Candida species. The efficacy of antibiotic therapy relies on the depth of understanding regarding the characteristics of the infecting organisms. Diverse techniques have been utilized for culturing the pathogens responsible for the sickness. [12] Nanotechnology has been utilized to create novel non-traditional antimicrobial agents known as Nano-antibiotics, which serve as effective treatments for infectious diseases, offering numerous advantages over conventional antibiotics, such as the lack of detrimental effects and enhanced efficacy against drug-resistant strains. Nano antibiotics exhibit antibacterial properties independently or enhance the efficacy and safety of conventional antibiotics, resulting in the establishment of elevated and effective local concentrations. [13,14] In our study out 50 samples 88% showed the growth of bacteria and Klebsiella pneumoniae was seen in 18% samples which was similar to previous results and the reason for the absence of growth in these samples may be due to the use of antibiotics before taking the sample, which reduces the percentage of bacterial growth, or the pathogen may be fungal or viral [15], and these results were similar with the results of Kuczkowskizz et al,[16] who found that 13.2% did not give bacterial growth. The results were similar with previous study conducted by Saranya et al, [17] and Basnet et al, [18] but Al-Mosawi [19] found that 9% of the isolates were K. pneumonia.In present study zinc oxide nanoparticles were produced using an aloe vera plant extract as a stabilizing and reducing agent. The synthesis of zinc oxide nanoparticles was demonstrated by the study's findings, which showed the creation of a precipitate at the reaction vessel's base. The primary advantages of employing Nano-zinc oxide biosynthesis include its cost-effectiveness, environmental safety, minimal danger, operational simplicity, and low toxicity.[20]Significant parallels and differences between the characterisation of zinc oxide nanoparticles (ZnO-NPs) produced chemically and biosynthetically were found, which is in good agreement with results from previous research. In UV-Vis spectroscopy, both types of nanoparticles showed a high absorption peak at about 370 nm, confirming the synthesis of ZnO with similar band-gap energies, in line with earlier research [21,22]. With estimated crystallite sizes of about 28 nm for chemically synthesized ZnO and 35 nm for biosynthesized ZnO—within the usual range reported in biosynthetic studies, where phytochemicals frequently act as capping agents influencing particle growth [23,24]—XRD analysis verified the hexagonal wurtzite crystal structure in both cases. Chemically produced ZnO-NPs were uniformly spherical, according to SEM imaging, but biosynthesized nanoparticles showed a variety of morphologies, including both spherical and rod-like geometries. This latter trait is typically ascribed to the presence of several phytochemicals in green synthesis [25,26]. This was corroborated by FTIR spectra, which showed functional groups such –OH, C=O, and Zn–O linkages in biosynthesized materials, confirming the participation of bio-organic molecules in stabilization [27, 28]. The elemental composition of Zn and O in both kinds was confirmed by EDX analysis, and the existence of residual plant-derived metabolites was confirmed by extra peaks for C and N in biosynthesized nanoparticles [26]. Both nanoparticle types maintained nanoscale dimensions with primarily spherical geometry, according to TEM imaging, which is consistent with observations from the literature [22]. Together, these findings show that although ZnO-NPs produced by the two synthesis routes have comparable structural and elemental characteristics, the biosynthetic approach provides a distinct profile enhanced with organic surface groups, resulting in morphological diversity and possibly improved functional qualities for biological or catalytic applications.The inhibitory effects of commercial and biosynthetic zinc nanoparticles at all concentrations on K. pneumoniae isolated from patients with otitis media

varied significantly. Our results are consistent with those of Rasha et al [29], who found that zinc nanoparticles biosynthesised from acacia plant extract inhibited the growth of K. pneumoniae bacteria. It also supports the results of Farzana and Iqra [30] who found that the zinc nanoparticle solution inhibited K. pneumonia.

## CONCLUSION

The biosynthetic approach utilizing Aloe vera extract yielded ZnO nanoparticles, as evidenced by UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), and energy-dispersive X-ray spectroscopy (EDX) analyses. Both nanoparticle forms possessed nanoscale dimensions and exhibited essential physicochemical characteristics; however, the use of phytochemicals as capping agents imparted greater morphological diversity to the biosynthesized ZnO-NPs. Nonetheless, chemically synthesized ZnO-NPs exhibited larger zones of inhibition and superior antibacterial efficacy across all concentrations. Both chemically manufactured and biosynthesized ZnO nanoparticles exhibit promising antibacterial capabilities, with the chemical variety showing enhanced efficacy against K. pneumoniae, while the biosynthesized variant provides ecological and biocompatibility advantages. Future research should enhance biosynthetic methods to augment antibacterial efficacy, investigate synergistic antibiotic combinations, and evaluate their in vivo safety and therapeutic potential in clinical otologic infections.

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